

The scientific contributions of Marianne Schiffer have been in the field of protein science: the structure and function of proteins, the determination of protein structures by x-ray diffraction, and the prediction of three dimensional structures based on the amino acid sequence.

**1. Representation of helical segments of proteins as “helical wheels”.**

The ‘helical wheel’ representation predicts helical segments from the amino acid sequence, and provides an understanding of the function of helical segments and of the positions of specific amino acids within the helix. The method is still widely used for the representation of helical peptides and helices in membrane proteins. It became so much a standard part of the field that the original 1967 paper is no longer referred to. The method is part of several computer packages for the analysis of primary structures, such as the widely used Wisconsin Package.

**2. Study of Immunoglobulins.**

Schiffer (in collaboration with A.B. Edmundson) determined the first high-resolution structure of an immunoglobulin fragment the Mcg light chain dimer at 3.5 Å resolution in 1973. On a sabbatical in Robert Huber’s laboratory at the Max-Planck Institute in Munich she determined the structures of the variable fragment from proteins Rei and Au. These proteins became the standards for understanding immunoglobulin structures and for modeling immunoglobulins.

To further understand the structures of light chains she determined additional structures by x-ray diffraction and made the unexpected discovery that the interactions of the variable domains and therefore the structure of the antibody binding site depends on the framework residues, as well as the variable ones, and on the solvent of crystallization.

In further studies of immunoglobulin light chains (in collaboration with F.J. Stevens) their work identified residues that effect thermodynamic stability. This work has relevance for amyloid fiber formation and for the making of super stable antibodies. Using site-specific mutagenesis they constructed light chain variable fragment whose stability improved by large factors (e.g. improved Delta G from 7.7 to 11.5 kcal/mol). This work made it possible to devise general methods for stabilizing antibodies and other proteins and study unstable mutants in the stable background.

Based on structural studies a new, previously unseen, variable domain interaction antibody light chains was discovered. This work demonstrated that the electrostatic potential of the protein surface and the unsatisfied hydrogen bonds at the protein-protein interface can determine protein-protein interactions.

The subgroups of alpha and beta chains of T-cell receptors were delineated and the subgroups were correlated with their functions (with T. T. Wu and E. Kabat).

**3. The study of the photosynthetic reaction center.**

Dr. Schiffer was the crystallographer in the group that made a determination of the structure of the photosynthetic reaction center from *Rhodobacter sphaeroides*; which was one of the first trans-membrane proteins for which three –dimensional structure was measured (with D. Tiede and J. Norris). This structure served as a model for all photosynthetic reaction centers from green plants that convert sun light into chemical energy and thus are responsible for most of life on earth. It also serves a model for other trans-membrane proteins.

The knowledge of the reaction center structure led Dr. Schiffer to the characterizations of the functions of tryptophan and proline residues in membrane proteins. Based on the structure, site-specific mutants were used to explore electron and proton transfer in the reaction center that led to the better understanding of these processes (with D. K. Hanson and P. Sebban). Second-site phenotypic revertants of the mutant reaction centers showed unexpectedly that the mutations that restore function can be 20 or more Å from the original mutation site. The structures of these mutant proteins are now being determined (with P.R. Pokkuluri) and found that correlated movement of a series of side-chains occur to repair the function destroyed by the original mutations by a mechanism they previously predicted.

#### **4. Mutiheme cytochromes from *Geobacter sulfurreducens*.**

Dr. Schiffer determined the structures of the critical *c7* cytochromes from *G. sulfurreducens* (with Y.Y. Londer and P. R. Pokkuluri). These cytochromes are involved in the reduction of iron and uranium by the bacteria in the subsurface environment. The three heme containing cytochrome *c7* is related to the more thoroughly studied cytochrome *c3* that contains four hemes from sulfate-reducing bacteria, it is characteristic of the family of Geobacteracea that dominate uranium contaminated sites. The structures of cytochrome *c7*, Ppca, the most abundant cytochrome in the periplasm of *G. sulfurreducens*, and that of four of its homologs were determined at high resolution and are being correlated with their individual functions determined in D. Lovley's laboratory. Based on the Ppca structure, 25 site-specific mutants of PpcA were designed; of these high resolution structures have already been determined for 10. The study of the mutants makes it possible to identify the specific residues that influence the reduction potential of hemes (with C. Salgueiro).

Dr. Schiffer identified novel polymers, two molecules that consist of four *c7*-type cytochrome domains (A, B, C, D) with 12 hemes, and another one with 9 domains and 27 hemes. The polymers are conserved in the related bacteria *G. metallireducens*. These polymers may act as “protein wires” to transport electrons in the periplasm. From one of the four-domain polymers the structures at high resolution of two pairs (A and B) and (C and D) domains together have been determined, as well as that of a single domain, C. The complete four-domain proteins have also been determined and it indicates that indeed “protein wire” is a good model for these molecules. Further, it was discovered that in each of the domains of the 4-domain and 9-domain polymers the heme coordination is unusual, for the first time both low and high-potential hemes are found in *c3* and *c7* cytochromes.